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Cancer Chemoprevention by Traditional Chinese Herbal Medicine and Dietary Phytochemicals: Targeting Nrf2-Mediated Oxidative Stress/Anti-Inflammatory Responses, Epigenetics, and Cancer Stem Cells

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ABSTRACT

Excessive oxidative stress induced by reactive oxygen species (ROS), reactive nitrogen species (RNS), and reactive metabolites of carcinogens alters cellular homeostasis, leading to genetic/epigenetic changes, genomic instability, neoplastic transformation, and cancer initiation/progression. As a protective mechanism against oxidative stress, antioxidant/detoxifying enzymes reduce these reactive species and protect normal cells from endo-/exogenous oxidative damage. The transcription factor nuclear factor-erythroid 2 p45 (NF-E2)-related factor 2 (Nrf2), a master regulator of the antioxidative stress response, plays a critical role in the expression of many cytoprotective enzymes, including NAD(P)H:quinine oxidoreductase (NQO1), heme oxygenase-1 (HO-1), UDP-glucuronosyltransferase (UGT), and glutathione *S*-transferase (GST). Recent studies demonstrated that many dietary phytochemicals derived from various vegetables, fruits, spices, and herbal medicines induce Nrf2-mediated antioxidant/detoxifying enzymes, restore aberrant epigenetic alterations, and eliminate cancer stem cells (CSCs). The Nrf2-mediated antioxidant response prevents many age-related diseases, including cancer. Owing to their fundamental contribution to carcinogenesis, epigenetic modifications and CSCs are novel targets of dietary phytochemicals and traditional Chinese herbal medicine (TCHM). In this review, we summarize cancer chemoprevention by dietary phytochemicals, including TCHM, which have great potential as a safer and more effective strategy for preventing cancer.

Key words: Cancer chemoprevention, Cancer stem cells, Epigenetics, Nrf2, Phytochemicals, Traditional Chinese herbal medicine

Oxidative stress and the antioxidant defense system

Hydroxyl peroxide, superoxide, and hydroxyl radicals, generally known as reactive oxygen species (ROS), are the metabolites of oxygen in normal cells, whereas nitrite, nitrate, and peroxynitrite, referred to as reactive nitrogen species (RNS), are the byproducts of nitric oxide (NO) metabolism.[1] Mitochondria-catalyzed electron transport reactions, UV light irradiation, X-rays, gamma rays, chronic inflammation, lipid peroxidation, and environmental pollutants are the common stimuli for ROS/RNS induction.[2,3] Maintaining a reasonable level of ROS/RNS in the body is essential for normal physiological processes, including cellular senescence and programmed cell death, which are beneficial anti-tumorigenic functions.[4,5] However, high levels of ROS/RNS generate oxidative stress, a critical trigger of genomic instability, defects in DNA

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damage repair, transformation of normal cells to premalignant cells, enhanced proliferation and survival of malignant cells, and subsequent cancer development.[6,7] Oxidative stress also has a significant association with many other chronic diseases such as neurodegenerative diseases [Alzheimer's disease (AD), Parkinson's disease (PD), and amyotrophic lateral sclerosis (ALS)], cardiovascular disease, diabetes, and inflammatory diseases.[5,8,9]

Non-enzymatic and enzymatic antioxidant regulation

Oxidative stress is counteracted by enzymes that inhibit the generation of ROS or by direct scavenging of free radicals by organic molecules.[5] Vitamin C (L-ascorbate), vitamin E, carotenoids, selenium, flavonoids, and thiol antioxidants such as glutathione, thioredoxin, and lipoic acid are the non-enzymatic antioxidants.[4,10,11] However, at high concentrations, some of these molecules, such as vitamin C and vitamin E, induce oxidative stress, thereby increasing ROS levels.[12]

Enzymatic antioxidants include superoxide dismutases (SODs), catalase, and glutathione peroxidase. Three isoforms of SODs (SOD1–SOD3) are the major antioxidant defense against ($O_2^{-}\cdot$), and all three isoforms require catalytic metals (Cu or Mn) for their activation.[13,14] The enzyme catalase degrades and reduces hydrogen peroxide.[15] Glutathione peroxidases include glutathione *S*-transferases (GSTs) and glutathione peroxidases (GPx), which are important for protecting the living organisms from free radical-induced oxidative damage.[16,17]

Phase I and phase II enzymes are closely associated with xenobiotic metabolism and are also involved in antioxidant activity. The phase I drug metabolic enzymes, which belong to the larger cytochrome P450 enzyme family, catalyze reactions through oxidation, reduction, hydrolysis, cyclization, and decyclization,. By contrast, phase II conjugating enzymes play crucial cytoprotective roles against carcinogens and ROS by catalyzing conjugation reactions involving glucuronic acid, sulfation, and glutathione to inactivate or detoxify harmful substrates by increasing their solubility or facilitating their excretion.[18-20] Most polyphenolic antioxidants exert their activity through phase II enzymes.[21]

Nrf2-related antioxidant regulation

When the cellular redox status of cells is altered by ROS/RNS, some ROS/RNS-sensitive regulatory transcription factors, such as nuclear factor-erythroid 2 p45 (NF-E2)-related factor 2 (Nrf2), nuclear factor-kappaB (NF-κB), and hypoxia-inducible factor-1alpha (HIF-1alpha), are modified with subsequent activation. Many phase II enzymes as well as some detoxifying genes, such as glutathione *S*-transferase (*GST*), peroxiredoxin1 (*Prx1*), γ-glutamate cysteine ligase (*γ-GCLC* and *γ-GCLM*), heme oxygenase-1 (*HO-1*), and NAD(P)H:quinine oxidoreductase (*NQO1*), are inducible and activated by Nrf2, a key orchestrator of antioxidant signaling.[1,20,22,23]

Nrf2 is a basic leucine zipper-containing transcription factor that activates phase II/detoxifying and many other genes through the cis antioxidant response element (ARE), which contains a conserved sequence (5′-A/G TGA C/T NNNGC A/G-3′, where N can be any nucleic acid).[24-30] Keap1 (Kelch-like ECH-associating protein 1), an interacting protein of Nrf2, serves as an adaptor that bridges Nrf2 and Cul3 for protein ubiquitination.[31] The sulfhydryl residues in Keap1 are sensitive to electrophiles, and ROS cause cellular redox status changes, making Keap1 a primary redox sensor,[32,33] although Nrf2 itself may also be a redox sensor that regulates its subcellular localization through its MES_{TA} motif.[34] [Figure 1] shows the schematic structure of Nrf2 and Keap1 and the mechanism of Nrf2 activation.

Nrf2-related inflammatory pathway regulation

Up to 20% of human cancer is triggered by chronic inflammation, and NF-κB is a key orchestrator of innate immune/inflammatory regulation.[35] With exposure to various stimuli, such as tumor necrosis factor alpha (TNF-α), interleukin (IL)-1, H_2O_2, lipopolysaccharide (LPS), or microbial infection, IκB proteins are subject to proteasome-mediated degradation as a consequence of phosphorylation at serine and threonine by IκB kinases (IKKs) within the IKK complex. [36,37] The degradation of IκBs leads to nuclear translocation of NF-κB to activate downstream target genes including different inflammatory cytokines and chemokines, adhesion molecules, enzymes [such as cyclooxygenase 2 (COX-2) and NO synthase], and many other stress response genes.[37-40] NF-κB activation has been observed in many cancer types. For example, suppression of the NF-κB pathway by deletion of IKKb, an upstream regulator of NF-κB, leads to inhibition of cancer cell proliferation and a dramatic decrease in tumor incidence in a colitis-associated cancer model.[41-44]

Potential interfaces and significant crosstalk are associated with Nrf2 and NF-κB signaling. Compared with wild-type mice, we and others have observed that in Nrf2-KO mice, inflammatory-related signals such as TNF, IL-1, COX-2, and iNOS attenuate expression in primary peritoneal macrophages upon stimulation with LPS after pretreatment with sulforaphane (SFN).[45,46]

Chemopreventive effects of phytochemical compounds

Phytochemicals possess potential anti-cancer effects

Dissecting the chemopreventive effects of dietary compounds and phytochemicals extracted from herbal medicines, particularly the mechanisms of their antioxidant activities, is an important area of research. For example, isothiocyanates, such as phenethyl isothiocyanate (PEITC) and SFN, have been purified from cruciferous vegetables, and other dietary compounds, such as curcumin and dibenzoylmethane (DBM), exhibit potential anti-cancer effects in transgenic adenocarcinoma of the mouse prostate (TRAMP) mice and the derivative tumor cell line TRAMP C1. Curcumin or PEITC, either alone or in combination, significantly decreases the incidence of prostate tumor formation.[47] Vitamin E is a generic name for structurally related tocopherols and tocotrienols. We and others have shown that gamma-tocopherol (gamma-T) enriched mixed tocopherol activates the expression of Nrf2 and suppresses prostate intraepithelial neoplasia (PIN) and tumor development in the TRAMP prostate cancer mouse model, corresponding to inhibition of the expression of proliferating cell nuclear antigen (PCNA), COX-2, and estrogen receptor α (ERα), and the induction of apoptosis.[48,49] Compounds identified in some herbal medicines also exhibit antioxidant activities. For example, the three common

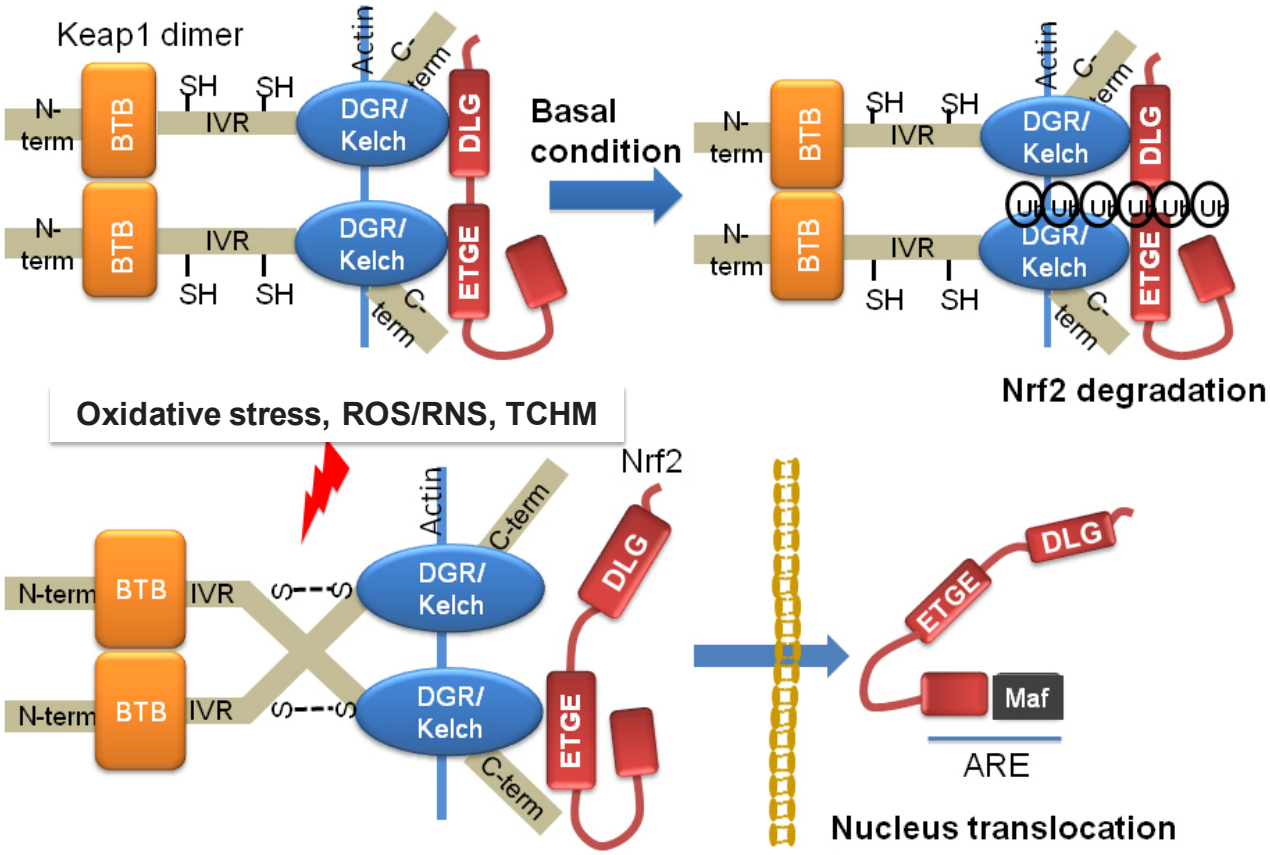

Figure 1. Hypothetical mechanism of Nrf2 activation.
Keap1 is dimerized through the BTB domain and is anchored to the actin cytoskeleton via the DGR/Kelch region. Nrf2 binds to the DGR/Kelch region of the Keap1 dimer via a high-affinity ETGE (hinge) motif and a low-affinity DLG (latch) bAU2 motif. The two-site binding exposes the Ub-acceptor site(s) in Nrf2. Under normal conditions, ubiquitinated Nrf2 is degraded by the proteasome, which maintains the equilibrium between synthesis and degradation of the Nrf2 protein in the cell. Once Keap1 is exposed to oxidants or electrophilic compounds, cysteine thiol groups in the IVR region of Keap1 interact with oxidative stress, inducing the formation of disulfide bonds. Disulfide bond formation results in a conformational change that renders Keap1 unable to bind to Nrf2, which then translocates to the nucleus. In this stage, the Ub-acceptor site is not easily accessible. The ubiquitination and proteasomal degradation of Nrf2 are impeded. The released Nrf2, in heterodimeric combination with other transcription factors such as small Maf, binds to the ARE regulatory region of phase II genes and enhances their transcription.

ginsenosides present in ginseng, Rb1 (Rb1), ginsenoside Rg1 (Rg1), and ginsenoside 20(*S*)-protopanaxatriol (20*S*), induce Nrf2 ARE in HepG2-C8 cells stably transfected with an ARE luciferase reporter gene.[50]

Potential antioxidant responses regulated by chemopreventive compound treatment

The potential mechanisms of these phytochemicals in chemoprevention may include 1) attenuating oxidative stress by serving as direct antioxidants or inducing Nrf2, as has been shown for curcumin, vitamin E, epigallocatechin-3-gallate (EGCG), and synthetic triterpenoid CDDO-Me;[51-56] 2) anti-inflammatory activities; and 3) cell cycle and apoptosis regulation. In HT-29 human colon cancers and RAW 264.7 murine macrophages, PEITC suppressed inflammation by inhibiting pro-inflammatory mediator mediators and cytokines (iNOS, COX-2, IL-1b, IL-6, and TNF-α). PEITC also suppressed LPS-induced phosphorylation and degradation of IkBa and decreased nuclear translocation of p65.[57] DBM, for example, blocks the growth and progression of prostate cancer in TRAMP mice and arrests TRAMP-C1 cells at the G2-M phase of the cell cycle. The expression of phosphorylated retinoblastoma, c-myc, cyclin D1, cyclin A, phosphorylated Akt, phosphorylated PDK-1, and phosphorylated S6 was also significantly reduced by DBM.[58]

Many of those phytochemicals activate multiple signaling pathways. The detailed molecular events of Nrf2 activation by various phytochemicals remain unclear. Mitogen-activated protein kinases (MAPKs), including extracellular signal-regulated kinases (ERKs), c-Jun amino-terminal kinases (JNKs), and protein 38 (p38), and other kinases such as phosphatidylinositol 3-kinase (PI3K) and protein kinase C (PKC) may play a role in this activation.[59-62] Phytochemicals such as SFN and PEITC induce the phosphorylation of ERK1/2, JNK1/2, and c-Jun.[63] ERK and JNK have positive effects on ARE-mediated activities and Nrf2 transactivation,[5,62,64,65] while phosphorylation of Nrf2 by p38 increases Keap1/Nrf2 binding and therefore inhibits Nrf2 activity.[66] PKC

Table 1. Examples of the effect of TCHM on the activation of Nrf2

TCHM name	Ingredient	Effect[1]	Concentration	Reference
Acanthopanax senticosus (刺五加 Cì Wǔ Jiā)	Aqueous extracts	↑GSH/GSSG ratio ↑Nrf2, ↑CuZnSOD, ↑MnSOD, ↑catalase, ↑GPx, and ↑GCLC against tert-butyl hydroperoxide induced oxidative stress in the livers of male Kunming mice	300 mg/kg, i.a.	[145]
Garlic (大蒜 Dà Suàn; the bulb of *Allium sativum*)	Diallyl sulfide (an organosulfur compound)	↑NQO1 and ↑Nrf2 in livers of male C57BL6/J wild-type mice	500 mg/kg, p.o.	[146]
Angelica Sinensis Radix (當歸 Dāng Guī)	Petroleum ether extract	↑NQO1 in Hepa-1c1c7 mouse hepatoma cells; ↑ARE-luciferase reporter activity in HepG2-ARE-C8 human hepatoma cells	5.5 ± 0.7 μg/mL	[147]
	Chloroform extract	↑NQO1 in Hepa-1c1c7 mouse hepatoma cells	3.9 ± 0.5 μg/mL	[147]
	Z-ligustilide (an alkylphthalide)	↑NQO1 in Hepa-1c1c7 mouse hepatoma cells; ↑ARE-luciferase reporter activity in HepG2-ARE-C8 human hepatoma cells; alkylated cysteine residues in human Keap1 protein	6.9 ± 1.9 μM	[147]
Dracocephali Rupestris Herba (嚴青蘭 Yán Qīng Lán; *Dracocephalum rupestre*)	Eriodictyol (a flavonoid)	↑Nuclear translocation of Nrf2, ↑HO-1, ↑γ-GCS and ↑intracellular glutathione against H_2O_2-induced oxidative stress in PC12 rat pheochromocytoma cells	80 μM	[148]
Ecklonia cava (昆布 Kūn Bù)	Eckol (a phlorotannin)	↑Erk, ↑Akt, ↑HO-1, and ↑phosphorylated form, nuclear translocation, ARE-binding, and transcriptional activity of Nrf2 in V79-4 Chinese hamster lung fibroblast cells	10 μg/mL	[149]
Ganoderma (靈芝 Líng Zhī; *Ganoderma lucidum*)	Water extract Ethanol extract	↑SOD, ↑catalase, ↑NQO1, ↑GSTP1 and ↑Nrf2 in OVCAR-3 ovarian cancer cells	10 μg/mL	[150]
Macleayae Herba cum Radice (博落回 Bó Luò Huí; *Macleaya cordata*)	Extract	↑HO-1 in RAW264.7 murine macrophage cells	50 μM	[151]
	Sanguinarine (an alkaloid)	↑Nuclear translocation of Nrf2 and ↑HO-1 in RAW264.7 murine macrophage cells	2 μM	[151]
Ginseng Radix (人參 Rén Shēn; the root of *Panax ginseng* C.A. Meyer)	Water extract	↑GSH content, ↑GST, ↓CYP1A1, ↑GSTA2, ↑GSTA3, ↑GSTM2, and ↑Nrf2 against benzo[alpha]pyrene -induced hepatotoxicity in SD rats	50 mg/kg/day, oral	[160]
	Ginsenoside 20(*S*)-Rg3 (a ginsenoside)	↑NQO1 and ↑nuclear translocation of Nrf2 against benzo[a]pyrene-induced DNA damage in neonatal human dermal fibroblasts	10 μM	[152]
	Ginsenoside Rb1, ginsenoside Rg1, and 20(*S*)-protopanaxatriol (ginsenosides)	↑Nrf2, ↑HO-1, and ↑ARE-luciferase reporter activity in HepG2-ARE-C8 human hepatoma cells	12.5 μM	[50]
	20(*S*)-protopanaxatriol (a ginsenoside)	↑Nrf2, ↑HO-1, ↑NQO1, and ↑UGT1A1 in murine prostate cancer TRAMP C1 cells	12.5 μM	[50]
Coptidis Rhizoma (黃連 Huáng Lián)	Berberine (an alkaloid)	↑Nuclear translocation of Nrf2, ↑Nrf2–DNA binding activity, and ↑HO-1-luciferase activity in rat brain astrocytes	10 μM	[153]
Rubi Fructus (覆盆子 Fù Pén Zǐ; the fruits of *Rubus coreanus*)	23-Hydroxytormentic acid (a triterpenoid glycoside)	↑GSH content, ↓MDA level, ↓ROS, ↑catalase, ↑SOD, and ↑nuclear translocation of Nrf2 against cisplatin-induced toxicity in renal epithelial LLC-PK_1 cells	50 μM	[154]
Scutellariae Radix (黃芩 Huáng Qín; the root of *Scutellaria baicalensis*)	Baicalein (a flavone)	↑Hepatic metabolic enzymes through Nrf2-mediated ARE pathway and ↑ARE-luciferase reporter activity in HepG2 human hepatoma cells	40 μM	[155]
Schisandrae Fructus (五味子 Wǔ Wèi Zǐ; the fruits of *Schisandra chinensis*)	Schisandrin B (a dibenzocyclooctadiene)	↑Nuclear translocation of Nrf2, ↑HO-1, ↑TrxR1, and ↑GCLC in lymphocytes	50 μM	[156]
Puerariae Radix (葛根 Gé Gēn; the root of *Pueraria lobata*)	Puerarin (an isoflavone glycoside)	↑Nuclear translocation of Nrf2, ↑HO-1, and ↑PI3K in Hepa-1c1c7 mouse hepatoma cells	100 μM	[157]
Salviae Miltiorrhizae Radix (丹參 Dān Shēn; the root of *Salvia miltiorrhiza*)	Extract	↑HO-1 and ↑nuclear translocation of Nrf2 in RAW 264.7 macrophages	10 μg/mL	[158]
	Tanshinone IIA (a diterpene)	↑GSH content, ↑NADPH, ↑G6PDH, ↑Nrf2, ↑ERK, and ↑PKB in TNF-α–treated human aortic smooth muscle cells (HASMCs)	5 μM	[159]

Table 1. Contd...

TCHM name	Ingredient	Effect[1]	Concentration	Reference
Four Agents Decoction (四物湯 Sì Wù Tāng)	A formula is composed of four herbs, Rehmanniae Radix Praeparata [熟地黃 Shú Dì Huáng; cooked rehmannia (root)], Angelica Sinensis Radix (當歸 Dāng Guī), Chuanxiong Rhizoma (川芎 Chuān Xiōng), and Paeoniae Radix (芍藥 Sháo Yào)	↑ARE-luciferase reporter activity and ↑Nrf2-regulated genes including *HMOX1*, *GCLC*, *GCLM*, *SLC7A11*, and *NQO1* in MCF-7 human breast cancer cells	2.56 mg/mL	[161]

[1]Abbreviation: ARE: antioxidant response element; CYP1A1: cytochrome P450 1A1; ERK: extracellular signal-regulated kinases; G6PDH: glucose 6-phosphate dehydrogenase; GCLC: glutamate-cysteine ligase catalytic subunit; GCLM: Glutamate-cysteine ligase modifier subunit; GPx: glutathione peroxidases; GSH: glutathione; GSSG: glutathione disulfide; GSTA2: glutathione S-transferase A2; GSTA3: glutathione S-transferase A3; GSTM2: glutathione S-transferase M2; GSTP1: glutathione S-transferase P1; HMOX1: heme oxygenase (decycling) 1; HO-1: heme oxygenase-1; MDA: malondialdehyde; NADPH: reduced nicotinamide adenine dinucleotide phosphate; NQO1: NAD(P)H:quinine oxidoreductase 1; Nrf2: nuclear factor-erythroid 2 p45 (NF-E2)-related factor 2; PI3K: phosphatidylinositol 3-kinase; PKB: Protein Kinase B (PKB); ROS: reactive oxygen species; SLC7A11: solute carrier 7A11; SOD: superoxide dismutases; TNF-α: tumor necrosis factor alpha; TrxR1: thioredoxin reductase 1; UGT1A1: UDP-glucuronosyltransferase 1A1; γ-GCS: γ-glutamylcysteine synthetase.

directly phosphorylates Nrf2 at serine 40,[60,67-69] and PI3K increases Nrf2 nuclear translocation.[61,70-72] However, when dissecting the phosphorylation sites of Nrf2 in detail, MAPK had only a slight effect on Nrf2 translocation and activity.[73] Thus, Keap1–Nrf2 signaling regulation by MAPK may be cell-type dependent, and the indirect effect on Keap1 or cofactors such as CBP may be more important in the regulation of this antioxidant pathway. [Table 1] shows the effects of various traditional Chinese herbal medicine (TCHM) on Nrf2 induction.

Future and novel targets for TCHM

Epigenetics

In recent years, evidence has shown that epigenetic alterations such as DNA methylation, histone modifications, and non-coding microRNAs (miRNAs) consistently contribute to carcinogenesis.[74,75] DNA methylation was the first epigenetic alteration observed in cancer cells and represents the most common molecular alteration in the origin of many cancers.[76,77] DNA methylation occurs at the 5′ position of the cytosine residue within CpG dinucleotides through the addition of a methyl group by DNA methyltransferases (DNMTs), including DNMT1, DNMT3A, and DNMT3B.[78,79] Although CpG dinucleotides are under-represented in the human genome, short regions rich in CpG content exist that are known as CpG islands, most of which are found in the proximal promoter regions of approximately half of human genes, where these CpG islands are generally unmethylated.[80] Thus, the hypermethylation of CpG islands leads to transcriptional silencing of tumor suppressors and other genes with important biological functions; global hypomethylation causes genomic instability and inappropriate activation of oncogenes and transposable elements.[81,82] In this context, many cancer-related genes, such as *hMLH1*, *MGMT* (DNA repair), $p16^{INK4a}$, $p15^{INK4b}$, $p14^{ARF}$ (cell cycle), death-associated protein kinase (*DAPK*) (apoptosis), *CDH1*, *CDH13* (cell cadherin), *Nrf2*, and *GSTP1* (detoxification) are inactivated by hypermethylation; genes such as *HRAS*, *CAGE*, *cyclin D2*, *maspin*, *MN/CA9*, *S100/A4*, *HPV16*, *14-3-3δ*, and *CT* are activated by hypomethylation.[76,78,83]

Histone modification is also commonly recognized as a cause of tumor-suppressor gene inactivation via the post-translational modifications (i.e. acetylation, methylation, phosphorylation, ubiquitination, sumoylation, and ADP-ribosylation) of the amino-terminal tails of histones.[84,85] The most common histone modifications are acetylation/deacetylation and methylation/demethylation, which are mediated by histone acetyltransferase (HAT) and histone deacetylase (HDAC) enzymes, respectively, in combination with histone variants and ATP-dependent chromatin remodeling.[86] Thus, HATs transfer acetyl groups from acetyl-CoA to the ε-amino group of lysine (K) residues in histone tails (open chromatin and gene activation), whereas HDACs remove histone acetyl groups by catalyzing their transfer to coenzyme A (CoA) (condensed chromatin and gene inactivation).[87] For instance, the loss of acetylated H4-lysine 16 (H4K16ac) as well as the overexpression of HDACs such as HDAC1, HDAC2, and HDAC6 has been commonly reported during tumorigenesis in various types of cancer.[88,89] Histone methylation occurs at lysine and arginine residues.[86] This mechanism is regulated by histone methyltransferases (HMTs) and demethylases (HDMs), leading to either activation or repression depending on the residues modified and the type of modification present.[75,90] Methylation of histone H3–lysine 4 (H3–K4), H3–K36, or H3–K79 is associated with transcriptionally active chromatin, whereas methylation of H3–K9, H3–K27, or H4–K20 is associated with transcriptionally repressed chromatin, the two main silencing mechanisms in mammalian cells.[74,91] In this context, cancer cells display widespread changes in histone methylation patterns, and changes in H3–K9 and H3–K27 methylation patterns have been observed in various forms of cancer.[86]

The miRNAs are small, endogenous non-coding RNAs (20–22 nucleotides) that are now recognized as an important component of epigenetic gene regulation in mammals, which control an array of cellular processes such as differentiation, development,

hematopoiesis, cell cycle regulation, and immunity.[92,93] Different cancer studies have shown that miRNAs interact with genes in diverse cellular pathways, resulting in differential gene expression profiles of normal and tumor tissues and among tumor types.[82,94] For instance, miRNAs such as miR-221 and miR-22 are highly expressed in different cancers (e.g. human thyroid, papillary carcinomas) targeting and down-regulating *p27* (Kip1). Likewise, the miR-17-92 oncogenic cluster targets *E2F1* (a cell cycle and apoptosis regulator), *BIM* (a pro-apoptotic gene that counteracts the anti-apoptotic activity of genes such as *BCL2*), and *PTEN* (a negative regulator of the oncogenic pro-survival PI3K/AKT signaling pathway) [[94]]. The down-regulation of *let-7* and miR-15/miR-16 miRNAs, which target the *RAS* and *BCL2* oncogenes, respectively, has also been described.[82] Another down-regulated miRNA is miR-126, which inhibits cancer cell growth, proliferation, adhesion, and invasion.[95] Other examples of miRNAs are miR-21, which is associated with tumor cell invasiveness and resistance to apoptosis, and miR-122, which is associated with tumor angiogenesis and cancer cell migration/invasion inhibition.[94,96]

Because epigenetic modifications are reversible, developing drugs that control epigenetic regulation represents a very promising and attractive avenue for treating or preventing cancers, including the development of functional foods or supplements as nutrition-based epigenetic modulators for cancer.[74,97] While HDAC (e.g. vorinostat, belinostat, romidepsin, and panobinostat) and DNMT (e.g. 5-azacitidine and 5-aza-20-deoxycytidine) inhibitors have been utilized at different phases of clinical trials,[98,99] the development of HDAC or DNMT inhibitors as anticancer drugs has been hindered by their adverse side effects.[75,100] However, many plant secondary metabolites extracted as natural products from fruits, vegetables, teas, spices, and traditional medicinal herbs have regulatory effects on the epigenetic machinery, thereby regulating multiple cancer-related pathways [e.g. *NFκB*, activator protein 1 (*AP-1*), signal transducers and activators of transcription (*STAT3*), *Nrf2*, peroxisome proliferator-activated receptor-γ (*PPARγ*), estrogen receptor, liver X receptor (*LXR*), and hypoxia inducible factor-1 (*HIF-1*)] and epigenetic cofactors (miRNAs) both *in vitro* and *in vivo*.[83,97,101]

Curcumin, which is found in turmeric, functions as a strong anticancer agent in different cancer models through the modulation of DNMT, HAT/HDAC, and miRNAs that target the *Nrf2*, *Neurog-1*, *RARβ2*, *PTEN*, and *P53* pathways.[102-105] Similarly, EGCG from green tea epigenetically controls several molecular cancer targets such as *RARβ*, *hTERT*, *GSTP1*, *p16*, *MGMT*, *hMLH1*, *MAGE-A1*, *Alu*, *LINE*, *BCLl2*, *IL-6*, *IL-12*, *NF-κB*, and *NOS-2* by DNA methylation, chromatin modification, and miRNA regulation.[87,106,107] Genistein from soybeans is another natural compound that controls the epigenetic machinery in many cancers both *in vitro* and *in vivo*. Genistein has been reported to be a DNMT inhibitor at the targets *p16*, *BCLl2*, *RARβ*, *MGMT*, *CDKN2A*, *GSTP1*, *HMGN5*, *BTG3* and *hTERT*, and *BRCA1*;[74,108,109] a chromatin modification inducer at *p21*, *p16*, *PTEN*, *CCLD*, *p53*, *FOXA3*, *SIRT1*, *BTG3*, *hTERT*, and *RARb*;[110,111] and an miRNA activator at *ZEB1*, *ZBTB10*, and *EGFR*.[112,113] Finally, isothiocyanates from broccoli, broccoli sprouts, and wasabi also exhibit broad effects on epigenetic mechanisms, such as DNMT inhibition activity at *GSTP1* and HDAC inhibition on *p21* and *GSTP1*.[114-116] Although these individual dietary phytochemicals have consistently shown great potential in the prevention and treatment of cancers, additional studies are still needed to elucidate the synergistic effects of the combined use of dietary components on the coordinated crosstalk between different molecular cellular pathways and epigenetic machinery as well as to analyze the safety profile of doses, route of administration, organ specificity, and bioavailability of these bioactive components in human clinical studies.[117]

Cancer stem cells

Tumors consist of phenotypically and functionally different subtypes of cancer cells that may have distinct origins at the time of tumor initiation.[118,119] Although the origins of cells that cause cancer are largely unknown, studies have speculated that a certain subset of cancer cells with the capability of self-renewal and continuous differentiation may be responsible for the growth and spread of tumors.[120,121] Many studies have demonstrated the existence of cancer stem cells (CSCs) in several human cancers. The CSC model asserts that a small distinct population of tumorigenic cells that is capable of self-renewal and perpetual proliferation initiates and develops cancer.[122,123] Tumors following the CSC model contain intrinsically different subpopulations of tumorigenic and nontumorigenic cells organized in a hierarchy.[124-129] Acute myeloid leukemia was the first cancer to support the CSC model. Only a small population of cells contributed to the formation of tumors when transplanted into immunocompromised mice. These leukemia-initiating cells were enriched for the specific surface marker profile $CD34^{+}CD38^{-}$.[124,125] Subsequent studies were performed to demonstrate the role of CSCs in solid tumor formation. In the same xenograft model, a very low density of cells presenting $CD44^{+}CD24^{-/low}$ initiated breast tumors.[126] Since these initial findings of the existence of CSCs were published, subsequent research has revealed evidence of CSCs in other human cancers such as colon, pancreatic, and ovarian cancer.[127,130,131] In the CSC model, there are two different types of cancer cells: tumorigenic and nontumorigenic. Through continuous self-renewal and differentiation, the minor population of tumorigenic CSCs gives rise to phenotypically diverse nontumorigenic cancer cells that are thought to compose the bulk of tumors but have little capacity to contribute to the progression of cancer.[120,132,133] Based on the CSC model, even though drug/radiation treatments result in the shrinkage of tumors, failure to eliminate tumorigenic CSCs may cause the recurrence of tumors because the few CSCs that survived from treatment can initiate the tumor again.[134,135] Thus, increasing evidence emphasizes the importance of the ability of both drugs and bioactive food components to modify the self-renewal capabilities of CSCs.[83,136]

Natural dietary compounds and TCHM targeting CSCs

Treatment of nasopharyngeal sphere-derived cells with (−) EGCG, a major bioactive compound in green tea, failed to inhibit growth and apoptosis but induced the formation of a sphere, suggesting that EGCG potently eliminates the stem cell character of nasopharyngeal cancer cells.[137] EGCG inhibits the self-renewing capacity of human prostate cancer cell lines (PC-3 and LNCaP)

containing a small population of CSCs presenting $CD44^+CD133^+$. Furthermore, EGCG also inhibits the self-renewing capacity of $CD44^+\alpha2\beta1+CD133^+$ CSCs isolated from human primary prostate tumors, as measured by spheroid formation in suspension. The inhibitory mechanism of EGCG on human prostate CSCs involves apoptosis, as was induced by activating caspase-3/7 and inhibiting the expression of Bcl-2, survivin, and XIAP.[138] Many studies have demonstrated the beneficial effect of cruciferous vegetables, such as broccoli and watercress, on cancer chemoprevention. SFN is a major active compound in cruciferous plants. It significantly decreases the growth of human pancreatic CSC-derived spheres by inhibiting the components of the sonic hedgehog (Shh) pathway and Gli transcription activity *in vitro*, suggesting the clonogenic depletion of the CSCs. SFN also inhibits downstream targets of Gli transcription by suppressing the expression of pluripotency-maintaining factors (Nanog and Oct-4) as well as PDGFRα and cyclin D1.[139] Treatment of a nonobese diabetic/severe-combined immunodeficient xenograft model with SFN inhibited the growth of breast CSCs and down-regulated the Wnt/β-catenin–related self-renewal pathway.[140] Curcumin is a well-known dietary phytochemical that is found in an Indian spice, turmeric, and has a large spectrum of chemoprevention activities. It suppresses mammosphere formation, reduces the proportion of aldehyde dehydrogenase-presenting cells, and inhibits Wnt signaling in breast stem/progenitor cells. However, curcumin is not toxic in differentiated cells, indicating that it could be a potential cancer prevention reagent for eliminating CSCs.[141]

Ginseng (人參 Rén Shēn; the root of *Panax ginseng*) is one of the best known Eastern traditional herbs, and research has demonstrated healthy beneficial properties of ginseng in humans. Ginsenoside F2, an active compound in ginseng that has been used in eastern Asia including Korea and China, induces apoptosis in breast CSCs via mitochondrial dysfunction. In addition, ginsenoside F2 induces the formation of acidic vesicular organelles, the recruitment of green fluorescent protein-light chain 3 (GFP-LC3)-II to autophagosomes, and elevation of Atg-7, suggesting that ginsenoside F2 initiates an autophagic progression in breast CSCs.[142] Celastrol, a triterpenoid from the plant Tripterygii Wilfordii Radix (雷公藤 Léi Gōng Téng; the root of *Tripterygium wilfordii*), effectively eradicated acute myeloid leukemia (AML) at the bulk, progenitor, and stem cell level, as demonstrated via chemical genomics methods such as gene expression-based high-throughput screening (GE-HTS) and the Connectivity Map.[143] Parthenolide (PTL) is a sesquiterpene lactone derived from the leaves of *Tanacetum parthenium* and is considered a main bioactive component in that herb. PTL induces the death of human leukemia stem cells *in vitro* without affecting normal hematopoietic cells.[144]

Many studies have been performed and are still ongoing to develop safer and more effective chemopreventive reagents. Owing to the critical role of CSCs in tumorigenesis, preventing the formation of or eliminating CSCs with dietary phytochemicals may be a safer and more efficient approach for combating strong malignancies. Thus, further studies to elucidate the physiological role of these dietary components in preventing the growth of CSCs are required.

CONCLUSIONS

In modern urbanized life, human beings are exposed to increased levels of various toxins, including environmental pollutants, dietary mutagens, carcinogens, microorganisms, and solar radiation. Accumulating evidence supports the effects of dietary phytochemicals, including TCHM, on ROS in health and diseases. Dietary phytochemicals have great potential not only for disease prevention, but also for improving the recovery from certain diseases and cancers by regulating various types of cellular damage caused by ROS. Dietary phytochemicals contribute to cellular protection by inducing phase II detoxifying/antioxidant enzymes such as GST, NAD(P)H quinine oxidoreductase 1 (NQO1), UDP-glucuronosyltransferase (UGT), and HO-1. Nrf2 plays an essential role in the transcriptional induction of phase II enzymes. Many studies have confirmed that various phytochemicals, including TCHM, contribute to cellular defensive mechanisms through the up-regulation of Nrf2. The restoration of various tumor-suppressor genes that are repressed by aberrant epigenetic alterations can be achieved by dietary phytochemical-induced epigenetic modifications. Studies of these natural compounds that modify the self-renewing capability and perpetual proliferation of CSCs have recently increased. Although the beneficial effects of dietary phytochemicals on human carcinogenesis are promising, effective, and safe, further studies of these natural dietary compounds are required. The elucidation of their biological functions, as well as their mechanisms of action, including which molecular targets in the signaling pathways are affected by phytochemicals, is needed to identify more effective and efficient chemopreventive solutions.

Keap1 is dimerized through the BTB domain and is anchored to the actin cytoskeleton via the DGR/Kelch region. Nrf2 binds to the DGR/Kelch region of the Keap1 dimer via a high-affinity ETGE (hinge) motif and a low-affinity DLG (latch) bAU2 motif. The two-site binding exposes the Ub-acceptor site(s) in Nrf2. Under normal conditions, ubiquitinated Nrf2 is degraded by the proteasome, which maintains the equilibrium between synthesis and degradation of the Nrf2 protein in the cell. Once Keap1 is exposed to oxidants or electrophilic compounds, cysteine thiol groups in the IVR region of Keap1 interact with oxidative stress, inducing the formation of disulfide bonds. Disulfide bond formation results in a conformational change that renders Keap1 unable to bind to Nrf2, which then translocates to the nucleus. In this stage, the Ub-acceptor site is not easily accessible. The ubiquitination and proteasomal degradation of Nrf2 are impeded. The released Nrf2, in heterodimeric combination with other transcription factors such as small Maf, binds to the ARE regulatory region of phase II genes and enhances their transcription.

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